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Signaling in the Third Dimension: The Peripodial Epithelium in Eye Disc Development

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Abstract

The eye-antennal imaginal disc of *Drosophila melanogaster* has often been described as an epithelial monolayer with complex signaling events playing out in two dimensions. However, the imaginal disc actually comprises two opposing epithelia (the peripodial epithelium, or PE, and the disc proper, or DP) separated by a lumen to form a sac-like structure. Recent studies expose complex molecular interactions between the PE and the DP, and reveal dynamic communication between the two tissues. Further findings suggest the PE makes important contributions to DP development by acting as a source of signaling molecules as well as cells. Here we summarize those findings and highlight implications for further research.

Introduction

The peripodial epithelium (PE) is a thin cell layer overlying and continuous with the disc proper (DP) of the developing imaginal disc in *Drosophila melanogaster*. Imaginal discs are larval tissues that give rise to the external structures of the adult, including the eye, antenna, wing, and leg. For much of the last 100 years the PE was thought to function primarily during metamorphosis in disc fusion and eversion - the processes by which the imaginal discs shift from flat internal epithelial structures to three dimensional external complex structures (reviewed by Fristrom and Fristrom, 1993). Most of the events in imaginal disc patterning and differentiation are presented in terms of signaling and gene function within the DP. During the past decade, researchers have applied molecular and genetic techniques to increase our understanding of the PE and the role it plays in normal tissue development. These works cumulatively reveal that the PE is a more complex and active tissue than previously appreciated. For example, the PE is required for DP cell survival and proliferation, and contributes directly to the cell complement of the imaginal disc proper (Cho et al., 2000; Gibson and Schubiger, 2000; Lim and Choi, 2004; McClure and Schubiger, 2005; Stultz et al., 2006).

Gene expression in the PE is dynamic and the PE is involved in regulating signaling pathways in the disc proper (Cho et al., 2000; Gibson and Schubiger, 2000). These results point to the need for careful consideration of the impact of the PE on the DP during our

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analyses of eye development and suggest that further study of gene expression and function within the PE itself may lead to a better understanding of *Drosophila* retinal development. Additionally, study of PE/DP interactions may provide new insights into the development of complex structures from other opposed layers such as those observed between the optic vesicle and the lens vesicle during vertebrate eye development or the mesenchyme and the apical ectodermal ridge during limb outgrowth (described in Gilbert, 2003). Within the context of eye development, there may also be similarities in signaling between the retinal pigment epithelia (RPE) and the neural retina. For example, signaling from the RPE supports the retina's growth, survival, and patterning, and in zebrafish the RPE contributes cells directly to the neural retina (Raymond and Jackson, 1995; Li et al., 2000).

Origin and morphogenesis of the DP and PE

Drosophila melanogaster is a holometabolous insect, and possesses three distinct forms following egg hatching: the juvenile larva, the pupa, and the adult imago. Holometabolous insects transition between the larval and adult forms through the process of metamorphosis. In *Drosophila*, larval development takes place over a period of 96 hours (at 25°C) between egg hatching and pupation. During this time, larvae progress through three stages, or instars, separated by molts (Figure 1). The juvenile larva contains internal epithelial structures that grow and develop to form the adult external structures. The progenitor tissues for the adult head, thorax, and genitals are referred to as imaginal discs (Bodentstein, 1950;Ferris, 1950).

Imaginal discs are initially set-aside during embryonic development. While experiments to establish the earliest time point of imaginal disc specification have conflicting results (reviewed by Cohen, 1993), it is clear that during the first larval instar the eye-antennal imaginal disc can be recovered as a distinct tissue of approximately 20-25 cells (Figure 1, orange). As indicated by their names, the wing, leg, and eye-antennal imaginal discs give rise to the corresponding external appendages. In addition, these discs contribute to the cuticle and body wall of the head and thorax (Fristrom and Fristrom, 1993). The eye-antennal discs also give rise to the dorsal photoperiodic organs called occelli.

The cells of the DP and PE are morphologically distinct (Figure 2) (McClure and Schubiger, 2005). PE cells have a broad, flattened squamous morphology (Figure 2C, E) while cells of the DP are tall and narrow with a columnar profile (Figure 2D, F). Finally, the short narrow cells at the border between the DP and PE layers are referred to as “cuboidal margin” cells (Figure 2B, blue arrow). The apical surfaces of the PE and DP are opposed and a lumen is formed in some areas of the disc between the two layers (Figure 2B) (Pallavi and Shashidhara, 2005). In the wing and leg, cuboidal margin cells are derived from both the DP and PE populations. However, in the eye all cuboidal margin cells are derived from the PE (Lim and Choi, 2004;McClure and Schubiger, 2005). Because the margin cells are derived from the PE lineage in the eye, the literature concerning their function will also be summarized in this review.

In addition to being morphologically distinct from the DP, clonal analysis reveals that the PE has molecularly defined, lineage restricted compartments. These compartment boundaries are displaced relative to those of the DP. For example, the Bolwig's nerve coincides with the dorsal/ventral (D/V) boundary of the eye PE, but this boundary is shifted ventrally from the optic stalk (OS, Figure 2A), which is the D/V boundary of the DP (McClure and Schubiger, 2005).

The PE contributes cells to the DP during larval development

Multiple lines of evidence suggest that the PE contributes cells to the final complement of the DP. Studies investigating the morphogenesis of imaginal discs have used cell counting at

different larval stages to quantify this contribution based on the difference between the number of cells observed in late third instar discs compared to the number expected based on observed rates of cell division. Cell counts obtained in the wing disc revealed approximately 1.7 DP cells observed per one cell expected if all cells were derived by proliferation in the DP alone. This led to the hypothesis that some DP cells are derived from the PE (Pallavi and Shashidhara, 2003; McClure and Schubiger, 2005). This hypothesis was confirmed by experiments using clonal analysis to trace the lineage of positively marked cells, demonstrating that the PE contributes cells directly to the DP during development. In contrast, the DP does not detectably contribute cells to the PE (Cho et al., 2000; Pallavi and Shashidhara, 2003; Lim and Choi, 2004; McClure and Schubiger, 2005). Comparison of these studies reveals that the potential for the PE to contribute cells to the DP may be temporally restricted. When clones are induced early, prior to lumen formation between the PE and DP, PE cells contribute to the DP as well as to margin cells. In contrast, PE clones induced after lumen formation only contribute to areas of the DP that are still in direct physical contact with the PE: DP-derived margin cell populations in the wing and DP regions physically in contact with cuboidal margin cells in the eye (Pallavi and Shashidhara, 2003; Lim and Choi, 2004). The mechanisms by which PE cells enter the DP population and assume a DP fate are unknown. However, this may occur via cell intercalation, a developmental process in which cells from one cell layer invade and acquire identity in an apposed layer, as observed in gastrulation and notochord formation (Reviewed by Pilot and Lecuit, 2005). An alternative hypothesis is that a fraction of PE cell divisions are within the plane of the PE while others are orthogonal to it. Upon division, daughter cells generated by orthogonal divisions may be inserted into the DP and assume DP fates. Planar versus orthogonal cell divisions are observed in several other developmental contexts including *Drosophila* neurogenesis, zebrafish spinal cord development, and rat retinal development (Cayouette et al., 2001; Geldmacher-Voss et al., 2003; Lee et al., 2006; Tawk et al., 2007). The localization of cell adhesion proteins that establish apico-basal cell polarity are important in determining the plane of cell division in these tissues, and examining their expression in PE cells during early larval development may help determine whether the same developmental process is occurring in the PE. Together, the cell count disparities and the clonal analyses show that the direct cell contribution from the PE to the DP during development is substantial and suggest that further study may provide new insights into organogenesis and how cells acquire new fates during intercalation.

Hedgehog and Wingless signaling from the PE are important for DP patterning and growth

The signaling of one tissue to pattern an apposed tissue is observed in many developmental contexts. For example, signaling from the notochord to neural tube cells and from the optic cup to the lens vesicle (described in Gilbert, 2003). In the developing eye-antennal imaginal disc, the major signaling molecules Hedgehog (Hh), Decapentaplegic (Dpp), and Wingless (Wg) are expressed throughout first and second instar in the PE but not in the DP, as detected by *lacZ* reporter expression (Cho et al., 2000). The mechanisms by which the dynamic and asymmetric patterns of morphogen expression are established remain unknown.

Loss- and gain-of-function experiments suggest that asymmetric expression of Hh, Wg, and Dpp from the PE during the second instar refines the expression patterns of the Notch ligands Delta (Dl) and Serrate (Ser), which are required for D/V boundary establishment (Figure 3A) (Cho et al., 2000). Notch signaling is activated at the D/V boundary in the eye disc prior to initiation of differentiation. Activation of Notch signaling is critical for disc growth and to establish the initiation point for differentiation at the posterior margin of the eye disc (Figure 3B) (Dominguez and de Celis, 1998; Papayannopoulos et al., 1998). Hh signaling in the PE is sufficient to induce expression of the Notch pathway ligand Serrate

(Ser) in the DP. Further, Hh signaling may be necessary during the establishment of the D/V boundary, because loss of Hh results in ubiquitous, rather than patterned, expression of Dl and Ser in the DP during the second instar (Cho et al., 2000). Both *wg* and *dpp* are necessary during second instar for the establishment of the dorsal DP compartment (Cho et al., 2000).

The Wingless signaling pathway has additional roles in the establishment of the D/V boundary of the developing eye (Cavodeassi, 1999). The Wg ligand is secreted from the dorsal and ventral margins of the DP, and the dorsal margin of the PE (Baker, 1988; Cavodeassi et al., 1999; Pereira et al., 2006). The GATA binding transcription factor Pannier (Pnr) was proposed to activate *wg* expression in the dorsal DP, making it the most upstream player identified in this pathway (Maurel-Zaffran and Treisman, 2000). However, *wg* expression is not lost in *Pnr* mutant clones in the DP. Rather, loss-of-function *Pnr* clones induced in the dorsal PE cause loss of a PE-specific reporter of *wg* expression (*2.11-LacZ*) (Pereira et al., 2006), suggesting that *Pnr* is necessary to drive *wg* expression in a small group of cells in the dorsal PE. Although the function of Wg secreted from these cells is unknown, it may serve to regulate both the initiation of differentiation and planar cell polarity in the DP as large loss-of function *Pnr* clones result in a failure of differentiation and loss of the eye, while small dorsal clones form ectopic D/V boundaries and have planar polarity defects (Maurel-Zaffran and Treisman, 2000). In addition to regulation by *Pnr*, *wg* expression driven by the PE enhancer is also regulated by the Jak-Stat pathway (Ekas et al., 2006). Jak-Stat signaling is involved in disc growth and is necessary to repress Wg expression to permit the initiation of differentiation (Tsai and Sun, 2004; Tsai et al., 2007). In addition, loss of Stat function phenotypes include loss of the eye or transformation of eye tissue to head cuticle (Ekas et al., 2006). These phenotypes are similar to those observed with *wg* or *Pnr* over-expression. Genetic data suggests that the Jak-Stat ligand Unpaired (Upd) is necessary and sufficient to repress *wg* in the PE (Figure 3C) (Ekas, et al., 2006). The shared aspect of the *Pnr* and *upd* loss-of-function phenotypes is a failure to initiate differentiation that is dependent on normal D/V boundary establishment. This leads to the hypothesis that a common target of these two pathways, Wg expressed from the dorsal PE, plays an important role in the normal establishment of the D/V boundary. Together with the regulation of *Ser* by PE-derived Hh during the second instar, these data support the conclusion that the PE is an important source of signals for D/V patterning in the developing eye disc.

PE-derived margin cells signal to the disc proper to regulate developmental processes

During eye disc development, PE cells give rise to a small band of cuboidal cells at the posterior margin of the eye that serve as a border between the DP and PE populations (Figure 2B, blue arrow). These cells are not only morphologically distinct but are also an important source of signaling to the developing DP. Margin cells function in DP dorso-ventral axis specification, DP proliferation and growth, initiation of differentiation, and the establishment of ommatidial chiral polarity during differentiation.

Cuboidal margin cells are an important signaling center for MF initiation—Disc margin cells play an important role in regulating the initiation of differentiation during eye imaginal disc development (Bras-Pereira et al., 2006). The *odd-skipped* gene family members *odd-skipped (odd)*, *drumstick (drm)*, and *brother of odd with entrails limited (bowl)* encode C2H2 zinc finger containing proteins that are expressed in posterior disc margin cells of the eye and together regulate the initiation of retinal differentiation. Bowl is predicted to function as a transcription factor. Odd and Drm share the conserved C2H2 domain with Bowl, but are not predicted to function as transcription factors. *bowl* expression in posterior eye disc margin cells is necessary during the late second instar to induce the expression of *hh* during initiation of the moving front of differentiation referred to as the

morphogenetic furrow (MF) (Figure 1, indigo line). However, *bowl* is not sufficient to induce ectopic *hh* expression in other parts of the disc, suggesting that other factors must act in collaboration with *bowl* to regulate *hh* during furrow initiation (Bras-Pereira et al., 2006).

Odd and Drm do not directly promote MF initiation, but rather refine the site of initiation. Odd and Drm are necessary to permit Bowl function by relieving repression of Bowl function by its inhibitor, Lines (Figure 3D) (Hatini et al., 2005; Bras-Pereira et al., 2006). Lines inhibits Bowl activity by binding to and destabilizing Bowl. Odd and Drm stabilize Bowl by binding to Lines and titrating it away from Bowl. *bowl* and *lines* are expressed widely in the disc, but Wg signaling restricts *odd* and *drm* expression in the eye disc to posterior margin cells (Figure 3C) (Bras-Pereira et al., 2006). Thus, Wg signaling indirectly restricts *bowl* function to the cuboidal margin cells of the posterior disc thereby restricting initiation of the MF (Bras-Pereira et al., 2006). The expression of *wg* is in turn repressed at the posterior margin by Jak-Stat signaling activated by Upd secreted from the center of the posterior margin (Figure 3C) (Ekas et al., 2006; Tsai et al., 2007). Upd is activated at the posterior midline by Notch signaling from the D/V boundary, and positively regulates proliferation in the second instar disc (Figure 3B) (Tsai and Sun, 2004). When these interactions are combined with the regulation of midline Notch activation by PE-derived Hh, Dpp, and Wg during the second instar (Cho et al., 2000) a genetic pathway emerges which connects early signaling events in the second instar in a cascade leading to the initiation of MF movement in the early third instar (Figure 3).

Cuboidal margin cells are a source of signals regulating planar polarity—

Signaling from PE-derived margin cells is also necessary during later patterning events in the eye imaginal disc. Following the initial differentiation of photoreceptors in the eye, ommatidial clusters undergo regulated rotation mediated by planar cell polarity pathways. The result of this rotation is that ommatidia are aligned with their neighbors, but with a mirror symmetry about the D/V axis (Figure 4). While the signal mediating this decision is unknown, the gene *dishevelled* (*dsh*), which encodes a membrane-bound protein that functions in both canonical and non-canonical Wg signaling, is required cell autonomously in margin cells to regulate long range cell polarity decisions in the disc proper (Lim and Choi, 2004). In discs where patches of the margin have been made mutant for *dsh*, ommatidia outside of the clone are incorrectly rotated. This function for Dsh in PE-derived margin cells is mediated, at least in part, by the canonical Wg pathway as loss of the downstream effector Armadillo (Arm) results in similar, though less severe, polarity defects. The differences in the Dsh and Arm loss-of-function phenotypes at the margin suggest that there may be additional undetermined pathways downstream of Dsh involved in producing the polarity signal (Lim and Choi, 2004). Within the DP, the signal for ommatidial rotation appears to be interpreted by non-canonical Dsh signaling which activates Jun kinase rather than Arm. Similar to loss-of-function effects, over-expression of Dsh in margin cells also causes changes in ommatidial polarity, suggesting that it is not the simple presence or absence of Dsh at the margin that is required for correct ommatidial rotation, but rather the correct relative levels of Dsh in the margin versus the disc proper that are required to correctly assign planar polarity (Lim and Choi, 2004). Together these results suggest that though the PE-derived cuboidal margin cells represent a small population, they uniquely integrate a host of signals to modulate disc growth and ommatidial polarity, and are key players in regulating the location and timing of MF initiation.

The lateral PE of the eye-antennal disc gives rise to ventral head structures

In addition to a direct contribution of cells to the DP, multiple studies reveal that the PE contributes significantly to external cuticular structures in the adult (Chouinard and Kaufman, 1991; Fristrom and Fristrom, 1993; Pilot and Lecuit, 2005; Stultz et al., 2006; Lee

et al., 2007). These structures include cuticle in the midline of the head (frons), the vibrissae, and ventral head structures including the maxillary palps (Figure 5, green text). A new mutation in a previously unidentified *cis*-regulatory element of *dpp* has been isolated that identifies a novel role for *dpp* in the PE (Stultz et al., 2006). This mutation ($dpp^{s\text{-}hc}$) results in a failure of ventral head formation and is located in an eye antennal disc enhancer 5' to the *dpp* gene. This enhancer regulates *dpp* expression in a pattern distinct from that of a previously characterized 3' disc enhancer (Figure 6). A reporter construct driven by the 5' enhancer (*SH53-LacZ*) is detected in the extreme lateral edges of the eye and antennal disc PE. The reporter is also active in adult animals and suggests that cells from the PE may contribute to the maxillary palps, vibrissae, and rostral membrane of the adult (Figure 5, green text) (Lee et al., 2007). In addition, another construct, *1096-GAL4*, drives *lacZ* in the dorsal and ventral disc PE, and in the adult cuticle surrounding the eye (Bessa and Casares, 2005). While it is possible that these enhancers are activated independently during the adult stage and that these *lacZ* positive cells are actually of DP origin, the most straightforward interpretation is that the cells of the lateral PE contribute to ventral head structures.

The pair-rule zinc finger transcription factor Odd-paired (Opa) was identified in a modifier screen as a regulator *dpp* expression from the 5' PE enhancer (Lee et al., 2007). This interaction is of interest because orthologs of these genes are associated with craniofacial malformations such as holoprosencephaly and the Dandy-Walker Complex in humans (reviewed in Grinberg and Millen, 2005). *opa* is necessary and sufficient to activate expression of the 5' *dpp* PE enhancer *SH53-lacZ* in the PE of the eye-antennal disc. Loss of o*pa* results in ventral head defects identical to those observed in $dpp^{sh\text{-}c}$ mutants. Supporting the model of genetic interaction between *opa* and *dpp,* compound heterozygote animals mutant for *opa* and *dpp* loss-of-function alleles phenocopy homozygotes for loss of either gene. Finally, *opa* reporters have also been used for lineage tracing in adult animals and are also expressed in ventral head structures which express *SH53-lacZ*, consistent with the interpretation that Opa regulates *dpp* in cells that give rise to structures of the ventral head (Lee et al., 2007). Together, these two studies reveal that cells of the lateral PE are likely to contribute substantially to the structures of the ventral head, including the maxillary palps and the vibrissae (Figure 5). Further, these data illustrate that signaling from a small region of the PE can have large consequences on adult eye and head structures. Finally, the regulatory interaction between Opa and Dpp may suggest a conserved network for head development during evolution, and may warrant further study as a model for congenital craniofacial malformations (Lee et al., 2007).

Communication between the PE and DP is bidirectional and may be mediated by long cellular processes

Dpp ligand expressed in the lateral PE is competent to signal within the PE as assayed using reporter expression for the target genes *brinker* (*brk*) and *daughters against dpp* (*dad*). The expression of both reporters changes in $dpp^{sh\text{-}c}$ homozygotes in accordance with previously published data in other tissues: *dad* is positively regulated by *dpp* and is lost in $dpp^{sh\text{-}c}$ PE, whereas *brk* is negatively regulated by *dpp* and its PE expression is expanded in $dpp^{sh\text{-}c}$ discs (Stultz et al., 2006). While Dpp from the PE signals within the PE and is essential for normal disc development, it does not appear to signal in the DP, since expression of the same reporters is unaltered in the DP of $dpp^{sh\text{-}c}$ discs (Stultz et al., 2006). This inability of Dpp from the PE to signal in the DP is also observed in the wing (Pallavi and Shashidhara, 2005). Paradoxically, while Dpp expressed in the PE does not signal directly to the DP, loss of Dpp from the lateral PE results in increased apoptosis in both tissue layers and loss of a region of head cuticle (gena, Figure 5) is observed in adults. Although the gena was classically considered to be derived from the DP, these results, coupled with the gena expression of *1096-GAL4* (Bessa and Casares, 2005), make it unclear whether loss of this

region is due to cell death within the PE or the DP. Nevertheless, the results of this study show that Dpp signaling is active within the PE, and suggest that it indirectly supports the growth and development of the DP. The mechanism of that support remains unclear since PE derived Dpp appears unable to signal directly within the DP (Stultz et al., 2006). This may reflect a structural and/or mechanical requirement for the PE in DP development, or activation by Dpp of another signal that is secreted from the lateral PE.

While Dpp produced in the PE of both the wing and the eye-antennal disc does not appear to signal to the DP, independent studies have found that the converse signaling event does take place in both the eye-antennal and wing discs. Dpp generated in the disc proper signals in the PE and is necessary for PE growth and survival (Gibson et al., 2002; McClure and Schubiger, 2005; Pallavi and Shashidhara, 2005). The mechanisms for ligand trafficking through the lumen to mediate signaling between the PE and DP are not fully understood. It is also not known what makes this signaling unidirectional. However, physical contacts between the PE and the DP may serve to traffic signals to cells separated by a lumen, as contacts between distant cells of the DP have previously been shown to transport ligands to activate signaling (elaborated below).

The nature of the contact between the PE and DP varies depending on the location in the disc. In some regions, the layers are directly apposed, whereas they are separated by a lumen in other parts of the discs. Microtubule filled processes, called translumenal extensions, have been identified that extend from PE cells across the lumen to contact the apical surfaces of DP cells in the eye, wing, leg, and haltere discs (Cho et al., 2000; Gibson and Schubiger, 2000; Lim and Choi, 2004). In the eye disc, translumenal extensions from the PE contact cells in the MF (Gibson and Schubiger, 2000). The MF is visualized as an apical constriction of cells and corresponds with cell cycle arrest, the onset of differentiation, and a flurry of signaling activity including Notch, Hedgehog, Epidermal Growth Factor Receptor, and Dpp. This indentation initiates at the posterior edge of the eye disc and progresses, wave-like, across the disc toward the anterior over the course of approximately two days (Figure 1, indigo line) (reviewed by Wolff and Ready, 1993; Pappu and Mardon, 2004). Loss of the PE or disruption of microtubule-based transport in translumenal extensions disrupts normal MF progression, the mitotic waves associated with MF progression, and normal patterning of ommatidia, suggesting that translumenal extensions mediate PE to DP communication necessary for normal MF progression (Gibson and Schubiger, 2000).

Translumenal extensions may mediate PE to DP communication by transmitting the Notch ligand Ser to the DP to modulate Notch activation in the MF. While *Ser* is activated in the DP during D/V boundary establishment in the second instar, by the third instar stage Ser is primarily expressed in the PE. *Ser* homozygous null mutant animals are rare and have small and disorganized eyes (Speicher et al., 1994). However, *Ser* loss-of-function clones generated by heat shock induced expression of FLP recombinase have no obvious phenotype in the adult eye or head (Tomlinson and Struhl, 1999; Singh and Choi, 2003). Confounding this paradox, ubiquitous ectopic expression of a dominant negative form of *Ser* during the first instar results in very strong reduction or loss of the eye, while ectopic expression during the second instar only causes loss of the ventral half of the eye (Singh and Choi, 2003). The differences between the ectopic expression and clonal analyses may lie in the roles of Ser in the PE. Ubiquitous expression of the dominant negative form of Ser will disrupt the PE as well as DP functions of Ser, whereas mitotic clones may be restricted to just one layer. During the third instar, expression of dominant negative Ser specifically in the PE results in flies with small eyes and abnormal ommatidial patterning, similar to the reported *Ser* homozygous null mutant phenotype (Gibson and Schubiger, 2000). Together, the PE specific over-expression phenotype, the localization of Ser in the PE, and the need for microtubule based transport in the translumenal extensions for normal DP growth and

furrow progression, suggest that the loss of *Ser* in the PE, rather than loss in the DP, is primarily responsible for the *Ser* phenotypes. Further, these results suggest that Notch signaling between the PE and DP may be important for normal eye development and may be mediated by translumenal extensions. PE-specific over-expression of the glycosyltransferase *fringe (fng)*, which mediates the preference of N for its ligand, produces phenotypes similar to the *Ser* dominant negative over-expression phenotypes (Gibson and Schubiger, 2000(Gibson and Schubiger, 2000). This further supports the interpretation that signaling from PE cells is affecting Notch activation in the DP. It is currently not known whether other pathways also use the translumenal extensions to mediate signaling across the lumen. Of particular interest is Dpp, which signals from the DP to the PE in both the wing and the antennal disc (Gibson et al., 2002; McClure and Schubiger, 2005; Pallavi and Shashidhara, 2005; Stultz et al., 2006).

Interestingly, long cellular processes have also been identified in cells of the lateral wing DP (Ramirez-Weber and Kornberg, 2000). These actin-containing processes are termed “cytonemes” and extend from DP cells located in the lateral disc to contact DP cells that secrete Dpp near the A/P boundary (Ramirez-Weber and Kornberg, 2000; Hsiung et al., 2005). Vesicles of internalized receptor-bound Dpp are transported in a retrograde fashion through the cytonemes toward distant cell bodies and are proposed to activate signaling there (Hsiung et al., 2005). The observed trafficking of ligand within cytonemes to distant cells within the DP leads to the hypothesis that translumenal extensions may also serve a similar function to traffic ligands generated in the DP to PE cells across the lumen. Whether bidirectional signaling is actually mediated by translumenal extensions remains to be determined.

Perspectives

Research conducted during the last decade has revealed that the peripodial epithelium is much more than a passive cover overlying the disc proper. In light of these findings, the PE should be considered more carefully during analysis of imaginal disc development. Analysis of gene function specifically within the PE is challenging, as many of the tools traditionally used to perform clonal analysis or to drive gene expression function in both layers. For example, heat shock induced clones may be induced in both layers, and many of the signaling pathways and retinal determination factors (such as *dpp*, *hh*, *wg*, *ey*, *eyes absent*, *sine oculis*, and *eyegone*) are expressed in both tissues. Some enhancer-based drivers such as *dpp-GAL4*, are expressed specifically in one layer; however, this is not always the case (e.g. *ey-GAL4*). For the eye and wing imaginal discs, a PE-specific *GAL4* driver has been identified, *c311-GAL4* (Manseau et al., 1997; Gibson and Schubiger, 2000). This driver can be used to ectopically express genes in the PE and has also been used in combination with *UAS-FLP* transgenes to specifically induce loss-of-function mitotic clones in the PE.

The PE is a dynamic and critical part of the eye-antennal imaginal disc, not only for the role it plays in the mechanical contortions of metamorphosis, but throughout development as a source of cells which contribute to the DP, signaling molecules that support and pattern the underlying disc, and direct contributions to adult structures. In addition, signaling is observed to flow bi-directionally between the two layers of the imaginal disc suggesting as yet unknown dimensions of communication and feedback. In particular, the mechanisms for communication between cells separated by a lumen remain unclear. Translumenal extensions are likely to transmit signals from the PE to the DP, including the Notch ligand Ser (Supported by Gibson and Schubiger, 2000). Dpp ligand generated in the DP appears to signal in the PE (Gibson et al., 2002; Pallavi and Shashidhara, 2005), although it is unknown whether translumenal extensions mediate this signal. Actin-based cytonemes appear to function as receiving antennas in lateral DP cells by facilitating rapid transfer of Dpp ligand

from Dpp secreting cells near the center of the DP, leading to the hypothesis that the microtubule based translumenal extensions of PE cells may function more like two-way radios, both sending and receiving signals.

A genome wide screen for genes involved in the “second mitotic wave,” the final, coordinated cell division of the eye-imaginal disc, revealed 29 genes that are differentially expressed in the PE in response to over-expression of the activated EGFR pathway ligand Spitz within the DP. Most of these genes are either novel or poorly characterized (Firth and Baker, 2007). These results suggest that we have only begun to understand the functions of PE cells. Further, some reconsideration of known genes may be in order. For example, what are the roles of *eyeless*, *optix*, and *eyes absent* in the PE? These known retinal determination factors are all expressed in the PE, which does not form retina, while the downstream gene *dachshund* is not. Understanding how these factors are regulated in the PE is likely to provide new insights into the combinatorial control of tissue specification.

Could study of PE development and the interplay between the PE and DP provide additional insights into mechanisms of vertebrate development? Indeed, an interesting model for a conserved regulatory network of ZIC and TGF-β signaling in metazoan head development has been proposed (Lee et al., 2007). Opa is the homolog of the vertebrate “Zinc finger of the cerebellum” or ZIC protein family, which have roles in myogenesis, neurogenesis, skeletal patterning, and left-right axis formation. Mutations in human *ZIC* genes are associated with cerebellar and cranio-facial malformation resulting in holoprosencephaly (HPE) and Dandy-Walker Complex phenotypes. HPE has variable and complex inheritance, which has led to the hypothesis that it may be subject to multigenic inheritance (reviewed by Ming and Muenke, 2002). In *Drosophila*, compound heterozygotes for *opa* and $dpp^{sh\text{-}c}$ mutations have reduced eye size and variable loss of ventral head structures (Lee, et al., 2007). The regulatory interactions between *dpp* and *opa*, the head phenotypes associated with their loss of function, and their strong genetic interaction suggests that regulation of head formation may be conserved in metazoans. Interestingly, mutations in human *SHH* and *SIX3* are also associated with holoprosencephaly (reviewed by Wallis and Muenke, 1999) and their homologs (*hedgehog* and *optix)* play important roles in eye imaginal disc development. Further research is necessary to determine if *Drosophila* head formation offers a succinct model for studying these complicated inherited developmental disorders (Lee et al., 2007). More generally, the tractability and accessibility of these tissues may provide an excellent system for understanding how opposed cell layers communicate and coordinate their growth and differentiation. We see similar opposition, communication, and coordination during multiple stages of vertebrate eye development including direct cell contribution to iris and corneal development by periocular mesenchyme, signaling between the optic vesicle and the developing lens, and the growth and patterning of the neural retina facilitated by the RPE (Raymond and Jackson, 1995; Cvekl and Tamm, 2004; Donner et al., 2006). Insights gained from the study of PE/DP interactions and communications may shed light not only on *Drosophila* retinal development, but also on developmental strategies employed in the complex assembly of the vertebrate eye.

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References

Baker NE. Transcription of the segment-polarity gene wingless in the imaginal discs of Drosophila, and the phenotype of a pupal-lethal wg mutation. Development. 1988; 102:489–497. [PubMed: 3181031]

Bessa J, Casares F. Restricted teashirt expression confers eye-specific responsiveness to Dpp and Wg signals during eye specification in Drosophila. Development. 2005; 132:5011–5020. [PubMed: 16221726]

Bodentstein, D. The Postembryonic development of Drosophila melanogaster. In: Demerec, M., editor. Biology of Drosophila. John Wiley and Sons, Inc.; New York: 1950. p. 275-367.

Bras-Pereira C, Bessa J, Casares F. Odd-skipped genes specify the signaling center that triggers retinogenesis in Drosophila. Development. 2006; 133:4145–4149. [PubMed: 17021046]

Cavodeassi F, Diez Del Corral R, Campuzano S, Dominguez M. Compartments and organising boundaries in the Drosophila eye: the role of the homeodomain Iroquois proteins. Development. 1999; 126:4933–4942. [PubMed: 10529412]

Cayouette M, Whitmore AV, Jeffery G, Raff M. Asymmetric segregation of Numb in retinal development and the influence of the pigmented epithelium. J Neurosci. 2001; 21:5643–5651. [PubMed: 11466435]

Cho KO, Chern J, Izaddoost S, Choi KW. Novel signaling from the peripodial membrane is essential for eye disc patterning in Drosophila. Cell. 2000; 103:331–342. [PubMed: 11057905]

Chouinard S, Kaufman TC. Control of expression of the homeotic labial (lab) locus of Drosophila melanogaster: evidence for both positive and negative autogenous regulation. Development. 1991; 113:1267–1280. [PubMed: 1687459]

Cohen, SM. Imaginal Disc Development. In: Bate, M.; Martinez-Arias, A., editors. The Development of Drosophila melanogaster. Cold Spring Harbor Laboratory Press: 1993. p. 747-841.

Cvekl A, Tamm ER. Anterior eye development and ocular mesenchyme: new insights from mouse models and human diseases. Bioessays. 2004; 26:374–386. [PubMed: 15057935]

Dominguez M, de Celis JF. A dorsal/ventral boundary established by Notch controls growth and polarity in the Drosophila eye. Nature. 1998; 396:276–278. [PubMed: 9834035]

Donner AL, Lachke SA, Maas RL. Lens induction in vertebrates: variations on a conserved theme of signaling events. Semin Cell Dev Biol. 2006; 17:676–685. [PubMed: 17164096]

Ekas LA, Baeg GH, Flaherty MS, Ayala-Camargo A, Bach EA. JAK/STAT signaling promotes regional specification by negatively regulating wingless expression in Drosophila. Development. 2006; 133:4721–4729. [PubMed: 17079268]

Ferris, GF. External Morphology of the Adult. In: Demerec, M., editor. Biology of Drosophila. John Wiley and Sons, Inc.; New York: 1950. p. 368-419.

Firth LC, Baker NE. Spitz from the retina regulates genes transcribed in the second mitotic wave, peripodial epithelium, glia and plasmatocytes of the Drosophila eye imaginal disc. Dev Biol. 2007; 307:521–538. [PubMed: 17553483]

Fristrom, D.; Fristrom, JW. The Metamorphic Development of the Adult Epidermis. In: Bate, M.; Martinez-Arias, A., editors. The Development of Drosophila melanogaster. Cold Spring Harbor Laboratory Press; 1993. p. 843-897.

Geldmacher-Voss B, Reugels AM, Pauls S, Campos-Ortega JA. A 90-degree rotation of the mitotic spindle changes the orientation of mitoses of zebrafish neuroepithelial cells. Development. 2003; 130:3767–3780. [PubMed: 12835393]

Gibson MC, Lehman DA, Schubiger G. Lumenal transmission of decapentaplegic in Drosophila imaginal discs. Dev Cell. 2002; 3:451–460. [PubMed: 12361606]

Gibson MC, Schubiger G. Peripodial cells regulate proliferation and patterning of Drosophila imaginal discs. Cell. 2000; 103:343–350. [PubMed: 11057906]

Gilbert, SF. Developmental Biology. Sinauer Associates, Inc.; Sunderland: 2003. p. 838

Grinberg I, Millen KJ. The ZIC gene family in development and disease. Clin Genet. 2005; 67:290–296. [PubMed: 15733262]

Hatini V, Green RB, Lengyel JA, Bray SJ, Dinardo S. The Drumstick/Lines/Bowl regulatory pathway links antagonistic Hedgehog and Wingless signaling inputs to epidermal cell differentiation. Genes Dev. 2005; 19:709–718. [PubMed: 15769943]

Hsiung F, Ramirez-Weber FA, Iwaki DD, Kornberg TB. Dependence of Drosophila wing imaginal disc cytonemes on Decapentaplegic. Nature. 2005; 437:560–563. [PubMed: 16177792]

Lee CY, Robinson KJ, Doe CQ. Lgl, Pins and aPKC regulate neuroblast self-renewal versus differentiation. Nature. 2006; 439:594–598. [PubMed: 16357871]

Lee H, Stultz BG, Hursh DA. The Zic family member, odd-paired, regulates the Drosophila BMP, decapentaplegic, during adult head development. Development. 2007; 134:1301–1310. [PubMed: 17329368]

Li Z, Joseph NM, Easter SS Jr. The morphogenesis of the zebrafish eye, including a fate map of the optic vesicle. Dev Dyn. 2000; 218:175–188. [PubMed: 10822269]

Lim J, Choi KW. Drosophila eye disc margin is a center for organizing long-range planar polarity. Genesis. 2004; 39:26–37. [PubMed: 15124224]

Manseau L, Baradaran A, Brower D, Budhu A, Elefant F, Phan H, Philp AV, Yang M, Glover D, Kaiser K, Palter K, Selleck S. GAL4 enhancer traps expressed in the embryo, larval brain, imaginal discs, and ovary of Drosophila. Dev Dyn. 1997; 209:310–322. [PubMed: 9215645]

Maurel-Zaffran C, Treisman JE. pannier acts upstream of wingless to direct dorsal eye disc development in Drosophila. Development. 2000; 127:1007–1016. [PubMed: 10662640]

McClure KD, Schubiger G. Developmental analysis and squamous morphogenesis of the peripodial epithelium in Drosophila imaginal discs. Development. 2005; 132:5033–5042. [PubMed: 16236766]

Ming JE, Muenke M. Multiple hits during early embryonic development: digenic diseases and holoprosencephaly. Am J Hum Genet. 2002; 71:1017–1032. [PubMed: 12395298]

Pallavi SK, Shashidhara LS. Egfr/Ras pathway mediates interactions between peripodial and disc proper cells in Drosophila wing discs. Development. 2003; 130:4931–4941. [PubMed: 12930782]

Pallavi SK, Shashidhara LS. Signaling interactions between squamous and columnar epithelia of the Drosophila wing disc. J Cell Sci. 2005; 118:3363–3370. [PubMed: 16079280]

Papayannopoulos V, Tomlinson A, Panin VM, Rauskolb C, Irvine KD. Dorsal-ventral signaling in the Drosophila eye. Science. 1998; 281:2031–2034. [PubMed: 9748163]

Pappu KS, Mardon G. Genetic control of retinal specification and determination in Drosophila. Int J Dev Biol. 2004; 48:913–924. [PubMed: 15558482]

Pereira PS, Pinho S, Johnson K, Couso JP, Casares F. A 3' cis-regulatory region controls wingless expression in the Drosophila eye and leg primordia. Dev Dyn. 2006; 235:225–234. [PubMed: 16261625]

Pilot F, Lecuit T. Compartmentalized morphogenesis in epithelia: from cell to tissue shape. Dev Dyn. 2005; 232:685–694. [PubMed: 15712202]

Ramirez-Weber FA, Kornberg TB. Signaling reaches to new dimensions in Drosophila imaginal discs. Cell. 2000; 103:189–192. [PubMed: 11057892]

Raymond SM, Jackson IJ. The retinal pigmented epithelium is required for development and maintenance of the mouse neural retina. Curr Biol. 1995; 5:1286–1295. [PubMed: 8574586]

Singh A, Choi KW. Initial state of the Drosophila eye before dorsoventral specification is equivalent to ventral. Development. 2003; 130:6351–6360. [PubMed: 14623824]

Speicher SA, Thomas U, Hinz U, Knust E. The Serrate locus of Drosophila and its role in morphogenesis of the wing imaginal discs: control of cell proliferation. Development. 1994; 120:535–544. [PubMed: 8162853]

Stultz BG, Lee H, Ramon K, Hursh DA. Decapentaplegic head capsule mutations disrupt novel peripodial expression controlling the morphogenesis of the Drosophila ventral head. Dev Biol. 2006; 296:329–339. [PubMed: 16814276]

Tawk M, Araya C, Lyons DA, Reugels AM, Girdler GC, Bayley PR, Hyde DR, Tada M, Clarke JD. A mirror-symmetric cell division that orchestrates neuroepithelial morphogenesis. Nature. 2007; 446:797–800. [PubMed: 17392791]

Tomlinson A, Struhl G. Decoding vectorial information from a gradient: sequential roles of the receptors Frizzled and Notch in establishing planar polarity in the Drosophila eye. Development. 1999; 126:5725–5738. [PubMed: 10572048]

Tsai YC, Sun YH. Long-range effect of upd, a ligand for Jak/STAT pathway, on cell cycle in Drosophila eye development. Genesis. 2004; 39:141–153. [PubMed: 15170700]

Tsai YC, Yao JG, Chen PH, Posakony JW, Barolo S, Kim J, Sun YH. Upd/Jak/STAT signaling represses wg transcription to allow initiation of morphogenetic furrow in Drosophila eye development. Dev Biol. 2007; 306:760–771. [PubMed: 17498684]

Wallis DE, Muenke M. Molecular mechanisms of holoprosencephaly. Mol Genet Metab. 1999; 68:126–138. [PubMed: 10527664]

Wolff, T.; Ready, DF. Pattern formation in the Drosophila Retina. In: Bate, M.; Martinez-Arias, A., editors. The Development of Drosophila melanogaster. Cold Spring Harbor Laboratory Press; 1993. p. 1277-1325.

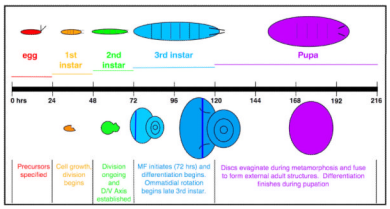

Figure 1. The eye-antennal imaginal disc is a dynamic structure in *Drosophila* development

The central black line depicts the passage of time in hours. *Drosophila* development is schematized to show the relative growth of the whole animal (above time line) and the eye-antennal disc (below time line). Key events for each stage are detailed in text (bottom). Embryonic development (red) takes place over 24 hours. During this time the eye-antennal imaginal disc precursors are specified. The eye-antennal disc is recoverable as a distinct structure during first instar (orange). At this stage the antennal disc is not distinct from the eye. During second instar (green), the disc proliferates, the D/V axis is specified, and the antennal disc becomes distinct. At the onset of third instar (blue) the MF initiates at the posterior margin (indigo line). The disc continues to grow and differentiation occurs progressively throughout the third instar. By late third instar the MF has crossed most of the eye field, and more posterior ommatidia are undergoing rotation. During pupation (violet) the antennal disc (not depicted) separates from the eye disc and both discs evert and fuse with head cuticle tissue and the labral and clypeolabral discs to produce the adult head. The final steps of differentiation are completed during pupation.

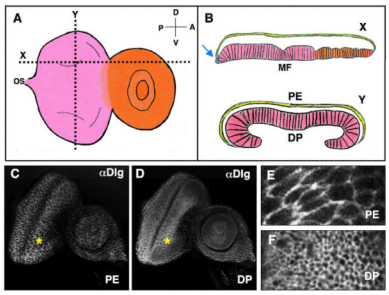

Figure 2. The squamous PE overlies the columnar DP in the eye imaginal disc

A) The eye-antennal imaginal disc is depicted at the level of the DP. A/P and D/V axes are indicated in the upper right corner. The eye disc proper (pink), the antennal disc proper (orange), and the optic stalk (OS) are illustrated. X and Y represent cross sections through the disc corresponding to the dashed lines. B) Sections of the disc corresponding to X and Y in panel A are depicted. The thin squamous PE (green) is shown overlying the DP (pink or orange), separated by a lumen. MF indicates the apical constriction corresponding to the morphogenetic furrow. The cuboidal margin cells are shown in blue (blue arrow). C-E) Mouse anti-Discs large staining (1:500, Developmental Studies Hybridoma Bank) labels septate junctions, revealing the apical cell membrane profile of eye-antennal imaginal disc cells. C) A thin optical section taken using confocal microscopy at the level of the PE reveals the large squamous cell profile. D) A thin optical section of the apical DP reveals the small profiles of columnar cells. The yellow asterisks in C and D mark the region of the disc magnified in E and F to facilitate comparison of the relative sizes of PE and DP cells.

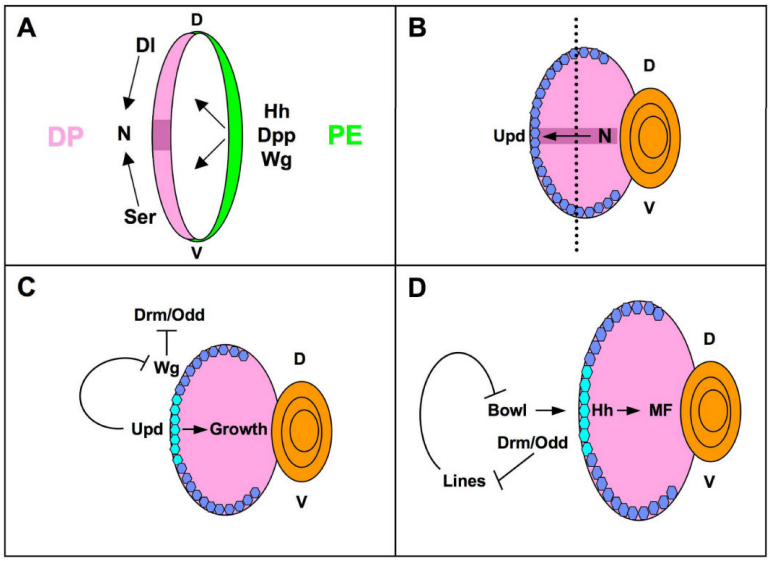

Figure 3. A model linking disc growth and D/V boundary establishment to initiation of the MF

The DP (pink), the PE (green), and the cuboidal margin cells (blue) are shown. In all panels dorsal (D) and ventral (V) are indicated, and posterior is to the left in B-D. Panel A is the cross section indicated by the dashed line in B. A) A second instar disc is represented in cross section to reveal the PE and DP. During second instar Dpp, Hh, and Wg are expressed asymmetrically within the PE (not shown) and their activity refines Dl and Ser expression in the DP. The boundary between Dl and Ser localization establishes activation of Notch at the midline and establishes the D/V boundary in the disc (dark pink). B) Activated Notch at the midline induces Upd expression in the posterior cuboidal margin cells. C) Upd secretion from the posterior margin stimulates DP growth and represses *wg* expression, particularly in the PE. Loss of Wg at the posterior margin results in expression of *odd* and *drm*. D) Drm and Odd inhibit Lines activity in posterior margin cells, de-repressing Bowl, which in turn activates the transcription of *hh*, initiating the MF.

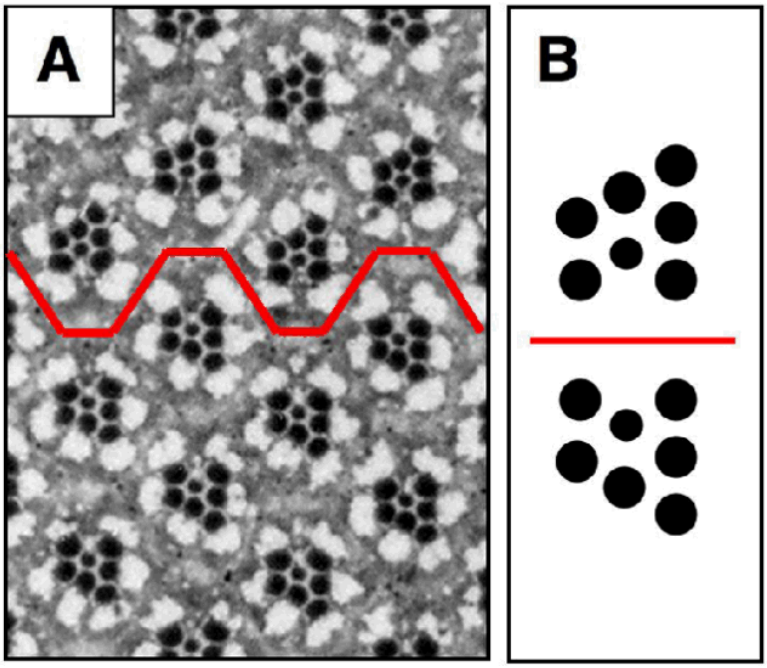

Figure 4. Ommatidia of the adult eye are arranged precisely and form a line of mirror symmetry about the D/V midline

A) A section through an adult eye reveals the highly ordered array of photoreceptors and ommatidia, and includes the D/V midline (red line). B) The diagram depicts the mirror image chirality that is a result of the integration of planar polarity cues. The solid red line represents the D/V midline or equator.

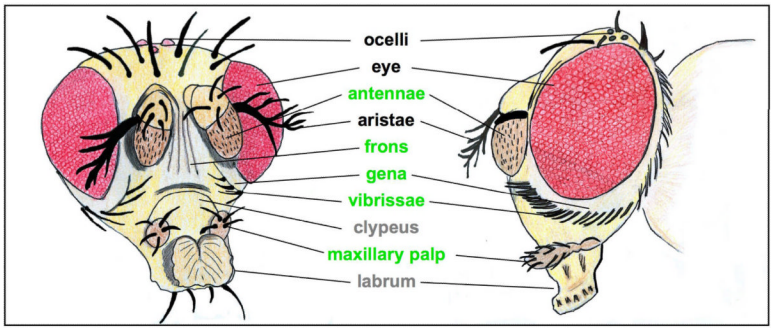

Figure 5. Anatomy of the adult head of *Drosophila melanogaster*

The anatomy of the fly head is depicted here for reference. Reporter analysis suggests that the cuticle beneath the eye, the second antennal segment, the vibrissae, maxillary palps, and the frons (names highlighted in green) contain cells that are derived from the PE. The clypeus is derived from the clypeolabral disc, and the labrellum is derived from the labial disc (names in gray).

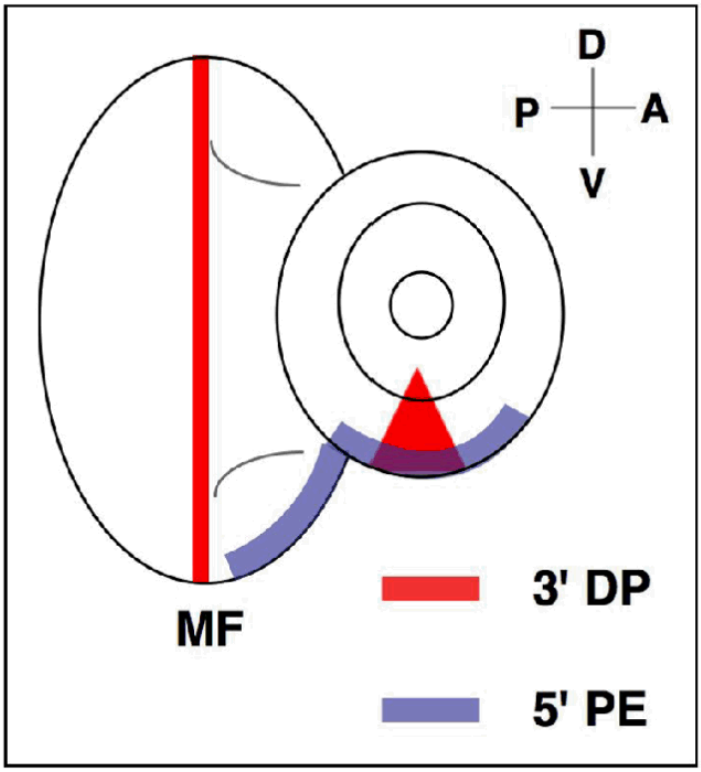

Figure 6. 5' and 3' *dpp* enhancers have distinct expression patterns

The 3' DP enhancer expression pattern is shown in red and the 5' PE enhancer is shown in blue. The expression of the 3' enhancer in the eye disc corresponds to the moving wave of differentiation, the morphogenetic furrow (MF).